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1 Highlights

- 2 • Acoustic cues of individuality and species were investigated in penguin contact calls.
- 3 • The source-filter theory is a valuable framework for studying bird calls.
- 4 • Contact calls of banded penguins encode individual identity information.
- 5 • Contact calls of congeneric *Spheniscus* penguins have diverged among species.

Vocal individuality and species divergence in the contact calls of banded penguins

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Abstract

Penguins produce contact calls to maintain social relationships and group cohesion. Such vocalisations have recently been demonstrated to encode individual identity information in the African penguin. Using a source-filter theory approach, we investigated whether acoustic cues of individuality can also be found in other *Spheniscus* penguins and the acoustic features of contact calls have diverged within this genus. We recorded vocalisations from two *ex-situ* colonies of Humboldt penguin and Magellanic penguin (sympatric and potentially interbreeding in the wild) and one *ex-situ* group of African penguins (allopatric although capable of interbreeding with the other two species in captivity). We measured 14 acoustic parameters from each vocalisation. These included temporal (duration), source-related (fundamental frequency, f_0), and filter-related (formants) parameters. They were then used to carry out a series of stepwise discriminant function analyses (with cross-validation) and General Linear Model comparisons. We showed that contact calls allow individual discrimination in two additional species of the genus *Spheniscus*. We also found that calls can be classified according to species in a manner far greater than that attributable by chance, even though there is limited genetic distance among African, Humboldt, and Magellanic penguins. Our results provide further evidence that the source-filter theory is a valuable framework for investigating the biologically meaningful information contained in bird vocalisations. Our findings also provide novel insights into penguin vocal communication and suggest that contact calls of the penguin family are affected by selection for individuality.

Keywords: bioacoustics; seabirds; source-filter theory; *Spheniscus*

36 1. Introduction

37 Penguins are a monophyletic group of pelagic seabirds that forage at sea and breed on land,
38 where most of the species form dense colonies (Williams, 1995; Schreiber & Burger, 2002). The
39 penguin genus *Spheniscus* comprises four living species collectively known as "banded penguins".
40 The genus can be further divided in two sister taxa: one formed by the Humboldt (*Spheniscus*
41 *humboldti*) and Galápagos (*S. mendiculus*) penguins and the second by the African (*S. demersus*)
42 and Magellanic (*S. magellanicus*) penguins. The two sister groups diverged in the Pacific and
43 Atlantic Oceans approximately 4 million years ago (Baker et al., 2006). However, despite their
44 genetic relationship, Magellanic and Humboldt penguins are partially sympatric and their
45 geographical distributions overlap along the Pacific Ocean coast of South America. For example,
46 in the Chilean islands of Puñihuil and Metalqui both species coexist in significant numbers
47 (Simeone and Schlatter, 1998; Simeone et al., 2009). By contrast, their closest relatives, African
48 and Galápagos penguins are allopatric and breed on the African continent and Galápagos
49 archipelago, respectively.

50

51 Penguins use vocalisations for individual localisation, recognition, and to maintain social
52 relationships and group cohesion (Jouventin, 1982; Favaro et al., 2014a). Based on the acoustic
53 properties of their vocalisations and behavioural context of emission, four categories can be
54 distinguished in the vocal repertoire of adult penguins (Jouventin, 1982): contact calls (uttered to
55 maintain cohesion with the group or the partner), agonistic calls (made during fights and in
56 territorial defence), as well as two types of display songs: complex vocalisations made of syllable
57 sequences that can be uttered by single birds (ecstatic display songs), or by pairs (mutual display
58 songs).

59

60 Penguin display songs are adapted for mate and parent-offspring recognition (Lengagne et al.,
61 1997; Lengagne et al., 2000; Aubin and Jouventin, 2002) and acoustic cues for individuality have
62 been found in all the penguin species that have been studied (Aubin and Jouventin, 2002; Aubin,
63 2004; Clark et al., 2006). The mechanisms used to encode the individual identity information in the

display songs are exposed to ecological sources of selection and also vary according to breeding ecology and the colonial lifestyles of the different species (Aubin, 2004). In particular, non-nesting species (e.g. King Penguin, *Aptenodytes patagonicus*) use the two-voice system as a principal means of identifying each other (Aubin et al., 2000), while in nesting species (e.g. Adélie penguin, *Pygoscelis adeliae* or Gentoo penguin, *Pygoscelis papua*), the pitch of the song, frequency and relative values of harmonics are the main cues for individual recognition (Jouventin and Aubin, 2002). Despite all the information available on display songs, very limited research has been directed toward studying the other call types, and in particular contact calls.

Contact calls have evolved as social signals to maintain cohesion in stable groups (Cheney et al., 1996; Kondo and Watanabe, 2009; Bergman and Sheehan, 2013), but also to advertise on the identity of the sender (Sharpe et al., 2013; Favaro et al., 2015), which is particularly important in fission-fusion societies (Ramos-Fernández, 2005; Terry et al., 2005; Janik et al., 2006). Moreover, contact calls can encode a great deal of information about the emitter. There is growing evidence that these vocalisations have the potential to provide information on sex (Guillette et al., 2010), age (Favaro et al., 2014b), group membership (Boeckle and Bugnyar, 2012), and even emotional state (Briefer et al., 2015). They can also contain acoustic cues to species (Gamba et al., 2012a; Cinkova and Policht, 2014) and population (Buhrmann-Deever et al., 2007; Husemann et al., 2014).

Although the African, Humboldt and Magellanic penguins are closely related, their ecstatic display songs are clearly recognisable even to human listeners (Jouventin, 1982). Indeed, this vocalisation has a significant amount of variation among species, and African and Magellanic penguins are more similar to each other than to Humboldt penguins (Thumser et al., 1996). Moreover, Thumser and Ficken (1998) showed that contact calls of *Spheniscus* penguins have very similar acoustic structure among the different species. They are short vocalizations with a clear harmonic structure, and that intraspecific variation only exists for the dominant frequency. However, these observations were based on a very limited number of individuals and vocalisations. A recent study showed that the contact calls of African penguin have the potential to allow individual

92 discrimination (Favaro et al., 2015). In particular, Favaro et al. (2015) used a Discriminant Function
93 Analysis (DFA) to show that contact calls in this species differ systematically between individuals
94 (DFA accuracy = 61.1%) and can be classified according to the emitter in a manner far greater
95 than that attributable to chance. Nevertheless, whether these vocalisations allow individual
96 discrimination also in other penguin species and contain other biologically meaningful information
97 still remains to be tested.

98

99 Bird calls are generated by vibrations of membranes in the syrinx (source, determining the
100 fundamental frequency, " f_0 ") and are subsequently filtered by the suprasyringeal vocal tract (filter,
101 resulting in amplified frequencies called "formants"). The source-filter theory of mammal vocal
102 production (Fant, 1969; Taylor and Reby, 2010) has recently been shown to be useful in gaining a
103 far greater understanding of individual identity information contained in seabird vocalisations
104 (Hardouin et al., 2014; Favaro et al., 2015). Following the source-filter theory approach, we
105 determined whether the contact calls of Magellanic and Humboldt penguins have the potential to
106 allow individual discrimination of the callers. Moreover, we tested whether the acoustic features of
107 contact calls have diverged across this genus. To this end, we compared calls of Magellanic and
108 Humboldt penguins (that potentially interbreed in the wild), and the African penguin, which is fully
109 allopatric.

110

111 **2. Methods**

112 **2.1 Ethics statement**

113 The research conforms to the Ethical Guidelines for the Conduct of Research on Animals by Zoos
114 and Aquariums (WAZA, 2005) and complies with all applicable Italian laws. Acoustic recordings
115 were non-invasive and we made every effort to minimize possible disturbance to the penguins.

116

117 **2.2 Study sites and penguins**

118 We recorded nine Magellanic penguins (5 males and 4 females) that were all the adult members
119 of an *ex-situ* colony housed at the Acquario di Genova, Italy. The colony was imported in 2006

120 from the SELWO Marina Delfinarium (Benalmadena, Spain), but was originally from Argentina
121 (wild individuals stranded due to an oil spill). In Genoa, the colony was maintained in a communal
122 indoor exhibit (123 m² including a pond of 66 m², water depth maximum 3 m) with three concrete
123 walls and one facing the visitor corridor made up of glass panels, which allows a combined vision
124 of open air and underwater penguin activity.

125
126 Vocalisations of Humboldt penguins were collected from six birds (3 males and 3 females)
127 belonging to a captive colony housed at the Acquario di Cattolica, Italy. The composition of the
128 whole colony was 4 males and 8 females. Penguins were housed in an indoor communal exhibit
129 of 75 m², including a pond of 35 m² (maximum depth 2 m). The colony was established at the
130 Acquario di Cattolica from 2007 to 2009 joining adult penguins from the Schönbrunn Zoo (Austria)
131 and the North of England Zoological Society, Chester (United Kingdom).

132
133 We recorded 24 adult African penguins (18 males and 6 females) from a large *ex-situ* colony (26
134 males, 27 females, 4 juveniles, and 3 chicks hatched during the study period), maintained at the
135 biopark Zoom Torino, Italy. The colony was established in 2009 by combining several adult African
136 penguins from four different zoological facilities in Europe (Artis Royal Zoo, Amsterdam, NL; Bird
137 Park Avifauna, Alphen an den Rijn, NL; Wilhelma Zoo, Stuttgart, DE; South Lake Wild Animal Park,
138 Manchester, UK). The colony was housed in an outdoor exhibit (1,500 m², including a pond of 120
139 m², water depth maximum 3 m), which reproduces the habitat of “Boulders Beach”, a natural
140 nesting site in South Africa.

141
142 All penguins recorded in this study were habituated to human presence and used to being audio-
143 and video-recorded during their normal daily activity. Additionally, all birds had a microchip
144 transponder and a flipper band to allow individual identification.

145

146 **2.3 Recordings of vocalisations**

Vocalisations of African penguins were collected over 50 non-consecutive days from September 2014 to March 2015. Recordings of Humboldt penguins were collected over 40 non-consecutive days between May and September 2015. Magellanic penguins were recorded over 30 non-consecutive days from February to April 2015. In all facilities, vocalisations were collected using the all occurrence animal sampling method (Altmann, 1974). Vocalisations were recorded at a distance of between 2 and 10 m from the caller with a RØDE NTG2 Super-Cardioid microphone (frequency response 20 Hz to 20 kHz, sensitivity -36dB +/- 2 dB re 1 V/Pa at 1 kHz, max SPL 131dB). In order to reduce recorded noise, the microphone was mounted on a RØDE PG2 Pistol Grip and protected with a windscreen. We also made every effort to orientate the microphone towards the calling bird. The microphone was connected to a TASCAM DR-680 or TASCAM DR-40 digital recorder (44.1 kHz sampling rate) and acoustic data were saved into an internal SD memory card in WAV format (16-bit amplitude resolution). All the files were then transferred to a laptop computer for later acoustic analyses.

160

161 **2.4 Acoustic analysis**

We used narrow-band spectrograms to visually inspect the overall spectral structure of audio recordings. In particular, the waveform and the FFT (Fast Fourier Transform) spectrogram were generated with the Praat (Boersma, 2001) sound editor window. We used a customised spectrogram setting (view range = 0 to 8000 Hz, window length = 0.02 s, dynamic range = 50 dB). For the purpose of this study we only selected contact calls (Supplementary Material, Figure S1). However, 355 calls for African penguins, 150 for Humboldt penguins, and 486 for Magellanic penguins were initially discarded because they showed excessive background noise or because calls were overlapping between different penguins vocalising at the same time. Overall, the spectrographic inspection allowed us to select a total of 392 vocalisations for African penguins, 119 for Humboldt penguins, and 976 for Magellanic penguins. The contribution of each penguin to the recordings is presented in the Supplementary Material (Table S1).

173

174 Acoustic measurements on selected vocalisations were carried out using a series of custom scripts

175 (see Gamba et al., 2012b; Favaro et al., 2015) in Praat v.5.4.08. The abbreviations that we use for
176 the various vocal parameters in this study are based on recent advice regarding this terminology
177 (Titze et al., 2015). For each contact call, we measured a series of spectral and temporal acoustic
178 parameters, which were potentially important to vocal distinctiveness. These included both
179 temporal measures, such as call duration (Dur), source-related vocal features (f_0) and filter-related
180 acoustic features (formants; Figure 1). We also quantified the mean harmonics-to-noise ratio value
181 (Sonority). However, before measuring formants, we estimated the approximate vocal tract length
182 (VTL) for banded penguins, to set a plausible number of formants in a given frequency range
183 (Gamba and Giacoma, 2006; Favaro et al., 2015). In particular, we built computational models of
184 the penguin vocal tract deriving information from cadavers of individuals died from natural causes
185 in different zoological facilities in Italy. We modelled vocal tract resonances using a MATLAB-
186 based computer program for vocal tract acoustic response calculation (VTAR, Vocal Tract Acoustic
187 Response; Zhou, 2004). The acoustic response of the vocal tract models and the visual inspection
188 of the spectrograms indicated 5 formants below 3500 Hz for the contact calls.

189
190 We extracted the f_0 contour of each call using a cross-correlation method [Sound: To Pitch (cc)
191 command]. Depending on the acoustic characteristics of each vocal type, we used a time step of
192 0.01 s, a pitch floor of 150 Hz, and a pitch ceiling of 350 Hz. From each extracted f_0 contour, we
193 obtained the frequency value of f_0 at the start (f_0 start) and at the end (f_0 end) of the call; the mean
194 (f_0 mean), minimum (f_0 min) and maximum (f_0 max) fundamental frequency values across the call.
195 We measured the percentage of duration from the beginning of the signal to the time at which the
196 minimum frequency (Time f_0 min) and the maximum frequency (Time f_0 max) occurs. Finally, we
197 extracted the contour of the first four formants (F_1 - F_4) of each call using a Linear Predictive Coding
198 analysis (LPC; Sound: To Formant (burg) command; time step = 0.045 s, maximum number of
199 formants = 5, maximum formant = 3500 Hz) and we calculated the average frequency values. In
200 addition, we calculated the formant dispersion (ΔF) using the methods described by Reby and
201 McComb (2003).

202

203 **2.5 Statistical Analysis**

204 We performed two separate cross-validated (leave-one-out) discriminant function analyses (DFA)
205 for Humboldt and Magellanic penguins to investigate whether contact calls could be used to
206 discriminate among individuals in these two species. In both analyses, the caller was used as the
207 group identifier and the acoustic variables as discriminant variables. We used a feed forward
208 procedure with default F-values threshold in SPSS v.20 for acceptance or rejection of the
209 discriminant variables. Moreover, the coefficients of classification were corrected according to the
210 group sizes, since the different individuals did not contribute equally to the samples. Finally, for
211 each vocal parameter, we calculated the Potential of Identity Coding (PIC) using the correction for
212 small samples (e.g. Charrier et al., 2004). PIC assesses the ratio between within-individual
213 variation and between-individual variation of an acoustic parameters (Mathevon et al., 2003). If the
214 ratio is > 1 , then the parameter has the potential to encode the individual identity information, since
215 its intra-individual variability is smaller than its inter-individual variability. Details for the PIC
216 calculation are presented in the Supplementary Material (Table S3).

217

218 We performed a cross-validated (leave-one-out) DFA to determine if contact calls of African,
219 Humboldt, and Magellanic penguins could be correctly classified to the correct species. In this
220 case, we used the species as test factor and the acoustic parameters as discriminant variables.
221 Moreover, since we did not record the same number of calls per individual, we also performed a
222 pDFA (Mundry and Sommer, 2007) for nested data with 1000 permutations. We used the species
223 as test factor and individuals as a control factor. Finally, since pDFA classification resulted in a
224 significant discrimination level ($p < 0.001$), we further investigated which vocal parameters
225 significantly differed in their average values among species using a series of univariate General
226 Linear Models (GLM) for ANOVA analysis. Specifically, each vocal parameter was entered in turn
227 as a dependent variable and the species was used as a fixed factor. Individuals were fitted as
228 random factors to control for repeated sampling. Finally, in order to control for individuality, we
229 used a nested design with individual factor nested under the dependent variable (species).

230

231 We performed pDFA analysis in R (R Core Team 2014, Version 3.2.2015-04-16, R Foundation for
232 Statistical Computing). All the other statistical analyses were performed in SPSS v.20 (IBM Corp.
233 Released 2013. IBM SPSS Statistics for Macintosh, Version 20.0. Armonk, NY: IBM Corp.).

234 **3. Results**

235 **3.1 Individual identity**

236 Using the source and filter vocal parameters as independent variables, the discriminant function
237 analysis (DFA) correctly classified 70.0% of the contact calls to the emitter for Magellanic penguins
238 and 60.5% for Humboldt penguins. The accuracy of the DFA decreased to 69.0% and 55.5%
239 respectively, when the more conservative leave-one-out cross-validation was applied. The
240 statistical significance of this classification and details of the canonical discriminant functions are
241 presented in Supplementary Material (Table S2). Overall, our results show that individual identity is
242 encoded also in contact calls of both Magellanic and Humboldt penguins. The PIC analysis
243 confirmed that both source- and filter-related components have the potential to encode individual
244 identity information (Supplementary Material, Table S3).

246 **3.2 Species recognition**

247 Calls were correctly classified to the correct species in 88.8% of cases. The accuracy of the DFA
248 decreased to 88.3% when the more conservative leave-one-out cross-validation was applied.
249 Table 1 shows values of the vocal parameters for contact calls of African, Magellanic and
250 Humboldt penguins. Details of the canonical discriminant functions generated for the discriminant
251 function analysis are presented in Supplementary Material (Table S4). Figure 2 shows that contact
252 calls of the three species form distinctive clusters in space, defined by discriminant functions 1 and
253 2. The nested pDFA confirmed that when controlling for individuality, contact calls could be
254 correctly classified on the basis of species (cross-validated classification after 1000 permutations:
255 expected = 41.38%, observed = 77.09%, $p < 0.001$). Results from GLMs (nested ANOVA, Table 2)
256 showed that average values of eight acoustic parameters significantly differed among species, and
257 thus are potentially important for vocal distinctiveness. These included call duration, four source-
258 (f_0 min, f_0 max, f_0 end, Time f_0 min) and three filter- (F_1 , F_4 , ΔF) related measures. Therefore, we
259 found evidence that penguin contact calls also encode species-specific acoustic signatures.

260 4. Discussion

261 We investigated whether banded penguin contact calls encode individual identity information and
262 species-specific vocal signature. We found evidence that contact calls of *Spheniscus* penguins
263 allow individual discrimination using both source- and filter-related components. Moreover, we
264 showed that contact calls can be classified according to species in a manner far greater than that
265 attributable by chance. Overall, our results provide further evidence that the “source-filter” theory of
266 vocal production (Fitch, 2010; Taylor and Reby, 2010) can be successfully applied to the
267 interpretation of information contained in bird vocalisations (Ohms et al., 2010; Budka and Osiejuk,
268 2013).

269

270 Penguin contact calls have been poorly studied compared to display songs. However, recent
271 research has found that the contact calls of African penguins are individually distinctive (Favaro et
272 al., 2015). In this species, the morphology and size of the vocal apparatus allow modifications of
273 the energy distribution across the spectrum. Accordingly, several source- and filter-related
274 vocalization features were found to exhibit a smaller amount of intra-individual variation when
275 compared to inter-individual variation (Favaro et al., 2015). Our results provide further evidence
276 that individual identity information is also encoded in contact calls of two other species of the
277 genus *Spheniscus*. The results of the PIC and DFA analyses also support the hypothesis that
278 vocal individuality in nesting penguins is determined by both source- and filter- related parameters.

279

280 Banded penguins often form flocks at sea for travelling and foraging (Wilson and Wilson, 1990).
281 When in flocks, they utter contact calls to maintain group cohesion (Jouventin, 1982). Penguins at
282 sea have highly synchronised diving behaviour (Siegfried et al., 1975), both when swimming to
283 travel (short and shallow dives of 10-20 seconds) and when diving for foraging (longest dives up to
284 2-3 minutes), (Wilson and Wilson, 1990). When underwater, birds are likely to get out of contact
285 with the other group members and, especially when in large flocks, they can surface
286 asynchronously (Wilson et al., 1986). In such circumstances, they need to call to locate other
287 group members when out of sight. In this fission-fusion context, where the effective distance of

288 visual signals is shorter compared to vocalisations, we expected to find high selective pressures
289 for vocal individuality (Janik and Slater, 1998). In more confined captive settings, juveniles banded
290 penguins swimming alone in ponds emit contact calls to maintain social contact with parents
291 (Thumser and Ficken, 1998). In all the colonies studied, we also observed adult pair members
292 keeping in touch with each other with contact calls when visually isolated and returning to the nest
293 after that the partner had repeatedly emitted contact calls (L. Favaro, personal observation).
294 Overall, our results support the hypothesis that penguin contact calls are social signals that have
295 evolved to facilitate social reunion and maintain group cohesion.

296

297 The ecstatic display songs of African, Magellanic and Humboldt penguins have diverged in several
298 spectral and temporal acoustic parameters (Thumser and Ficken, 1998) and it has been suggested
299 that this vocal type can reflect phylogenetic relationships within this genus (Thumser et al., 1996).
300 Our DFA analysis results support the hypothesis, demonstrating that acoustic cues to species are
301 also present in contact calls and are likely to depend on the anatomy of the vocal tract. In addition,
302 the GLM underlined how eight source- and filter- vocal parameters differ among the three species.
303 However, in closely related seabirds, the ability to detect species-specific vocal features is
304 controversial (Bretagnolle and Robisson, 1991; Cureé et al., 2010; Curé et al., 2012) and there is
305 no evidence that penguins are capable of using such information from vocalisations. In particular,
306 penguins of the genus *Spheniscus* have limited phylogenetic distance and hybrids have been
307 found where the geographical distributions of Magellanic and Humboldt penguins overlap along the
308 Pacific coast of South America (Simeone and Schlatter, 1998; Simeone et al., 2009). This
309 suggests that the species-specific recognition systems fail to prevent heterospecific confusion.
310 Banded penguins are also known to readily hybridise when grouped together in captivity
311 (McCarthy, 2006). In sympatric closely-related species, differences in signalling characters often
312 evolve to prevent incorrect mate choice (Losos and Leal, 2013). However, although we found
313 acoustic differences in both sympatric and allopatric penguins within the penguin genus
314 *Spheniscus*, we suggest that species-specific acoustic signature in their contact calls is more likely
315 to be a by-product of divergent ecological selection rather than a pre-zygotic mechanism to prevent

316 hybridization. In social animals, not all the acoustic cues encoded in calls are necessarily salient to
317 receivers (Townsend et al., 2011). We recommend additional studies using playback experiments,
318 to further investigate the sensitivity of penguins to acoustic cues of species.

319
320 In conclusion, our findings confirm that the source-filter theory of vocal production can be
321 successfully adopted to study bird vocalisations. Using this approach, we provide further evidence
322 that banded penguin contact calls encode individual identity and species-specific signatures. We
323 suggest that the high levels of individuality in these vocalisations are an adaptation to travelling
324 and foraging in large flocks at sea.

325

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333

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454 **Table 1.** Values of the vocal parameters (mean \pm SD) for the contact calls of each penguin species.

Vocal parameter	African[*] (n = 392)	Humboldt^{**} (n = 119)	Magellanic^{***} (n = 976)
Dur (s)	0.57 \pm 0.12	0.91 \pm 0.20	0.86 \pm 0.16
f_0 mean (Hz)	275 \pm 22	253 \pm 16	270 \pm 23
f_0 max (Hz)	299 \pm 21	302 \pm 22	291 \pm 21
f_0 min (Hz)	248 \pm 25	222 \pm 20	246 \pm 23
f_0 start (Hz)	267 \pm 29	275 \pm 29	267 \pm 25
f_0 end (Hz)	279 \pm 28	278 \pm 27	267 \pm 25
Time f_0 min (%)	69 \pm 34	50 \pm 26	51 \pm 40
Time f_0 max (%)	41 \pm 34	49 \pm 43	39 \pm 35
Sonority	8.63 \pm 5.98	4.9 \pm 3.54	7.43 \pm 3.02
F ₁ (Hz)	572 \pm 133	450 \pm 109	599 \pm 80
F ₂ (Hz)	1030 \pm 143	976 \pm 96	978 \pm 88
F ₃ (Hz)	1668 \pm 200	1660 \pm 123	1625 \pm 118
F ₄ (Hz)	2468 \pm 230	2475 \pm 148	2431 \pm 98
ΔF	697 \pm 65	691 \pm 45	683 \pm 31

455 * 24 individuals; ** 6 individuals; *** 9 individuals

456 **Table 2.** GLM results (ANOVA analysis) for the effects of penguin species on the contact call
 457 acoustic variables.

Vocal parameter	df	F	Significance
Dur	2	30.08	p < 0.001
f_0 mean	2	2.73	ns
f_0 max	2	3.90	p < 0.05
f_0 min	2	4.04	p < 0.05
f_0 start	2	1.28	ns
f_0 end	2	5.46	p < 0.05
Time f_0 min	2	4.27	p < 0.05
Time f_0 max	2	1.18	ns
Sonority	2	1.75	ns
F ₁	2	14.07	p < 0.001
F ₂	2	2.46	ns
F ₃	2	2.52	ns
F ₄	2	3.51	p < 0.05
ΔF	2	3.27	p < 0.05

458 Identity was nested into species and included as a random factor. Species was included as a fixed
 459 factor.

460 **Figure captions**

461

462 **Figure 1.** Spectrogram (Gaussian window shape, view range = 0 to 5000 Hz, window length =
463 0.05 s, dynamic range = 70 dB, time step = 0.004 s, frequency step = 20 Hz) and LPC spectrum
464 (500 Hz cepstral smoothed) of contact call (African penguin) showing f_0 and formants (F_1 - F_4).

465

466 **Figure 2.** Discriminant scores generated by the discriminant functions 1 and 2 to classify contact
467 calls of African (*S. demersus*), Humboldt (*S. humboldti*), and Magellanic (*S. magellanicus*)
468 penguins. Black dots indicate the centroid of each species.

Figure 1
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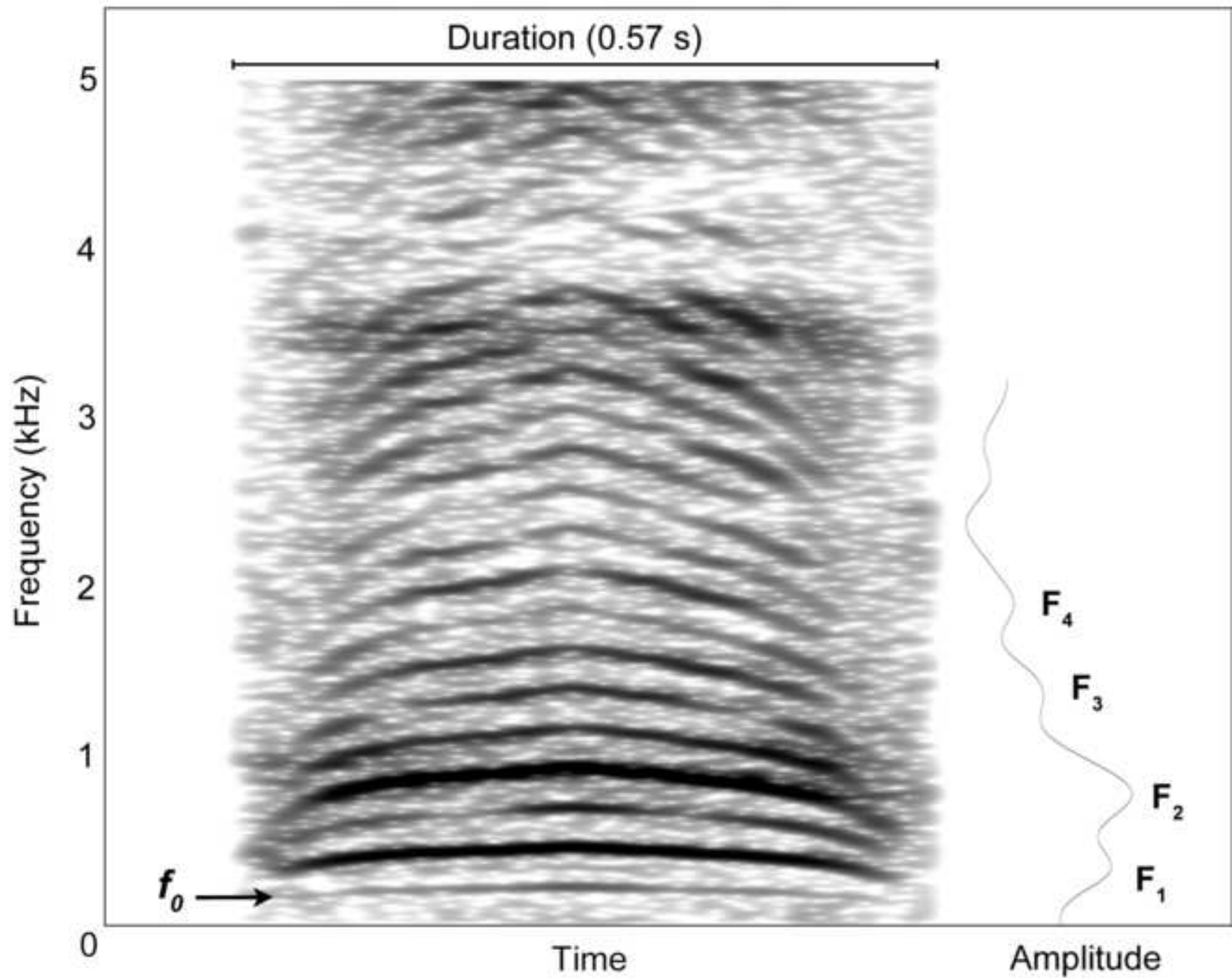
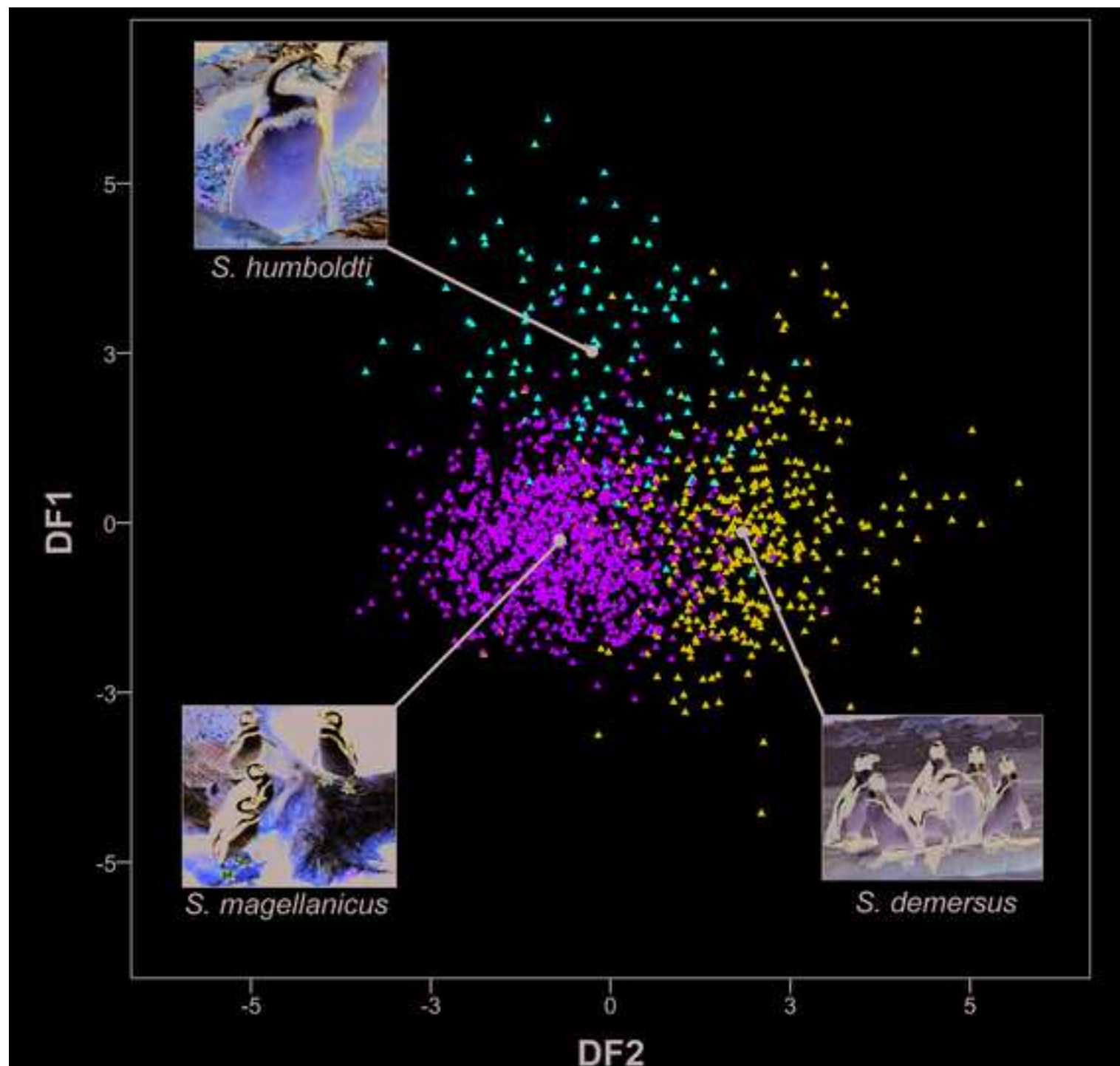


Figure 2
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